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Nutritive Analysis of *Azolla pinnata* and its Cultivation during Winter Season

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A B S T R A C T

The experiment was conducted to evaluate the nutritional composition of *Azolla pinnata* and its growth during winter stress as a livestock feed along with its propagation. The study was conducted at National dairy research institute Karnal on Azolla cultivation pits covered with high-density polyethylene (HDPE) sheets during winter season and the mean level of Temperature humidity index was below 72 in the range of (68-70). After its full growth, *Azolla pinnata* was harvested, washed properly to remove smell and dried in sun. The sun dried Azolla was further used for proximate estimation and mineral analysis. The average percentage of DM content, organic matter %, crude protein, ether extract, ADF and NDF calculated during trial was 9.90, 79.7, 26.5, 3.9, 39.4, and 44.28 (% DM Basis) .The total ash content on DM % basis recorded was 20.3 %. The proximate analysis proved that Azolla is a rich source of protein and mineral profiling of *Azolla pinnata* in terms of calcium, phosphorous, potassium was 1.67%, 0.31% and 2.68% respectively (on percent DMB). From this study, it may be concluded that *Azolla pinnata* had high crude protein content and better mineral profiling so it can act as a potential protein supplement in the feed of livestock.

Introduction

The term Azolla combines two Greek words azo (to dry) and allyo (to kill), reflecting plants failure to thrive under dry circumstances (Lumpkin and Plucknet 1982). It is a free-floating aquatic fern belonging to the Salviniaceae family and the genus Azolla. *Azolla pinnata* is the most commonly dispersed species in both tropical and temperate regions in India and around the globe. Due to high pressure on land to produce human food plants for the growing human population, it leaves little land accessible for forage cultivation or feed production to feed livestock. Using marketable feed is not always cost-effective, and it is shrinking the net income of

producers. So in order to bridge this huge gap between demand and nutrient supply and guarantee optimum livestock production throughout the year, the use of non-conventional feed resources as a complement or replacement for standard feed can be an effective method. Azolla is protein-rich that can be used for animal nutrition as a supplier of plant protein and provitamins (Lejeune *et al.*, 2000).

Azolla can act as a valuable green feed supplement for dairy cattle to improve productivity (Chatterjee *et al.*, 2013). Due to easy cultivation, good nutritive value and high productivity it can be used as a beneficial fodder supplement as revealed by various researchers (Singh and Subhudhi 1978, Prabina and Kumar 2010). Thus, the present study was conducted to analyse the nutritive value and mineral profiling of *Azolla pinnata*

Materials and Methods

The present research work has been carried out at Livestock Production Management Division, ICAR-National Dairy Research Institute, Karnal.

Cultivation and propagation of *Azolla pinnata*

Azolla has been propagated at the Azolla Production Unit established at Fodder research centre, near ABRC, NDRI, Karnal. A rectangular pit of size 11.0*4.5 feet (L*B) and 90 cm depth was prepared (Total 19 pits). Then the pits were covered with the silpauline sheets and surface of the pit was made uniform. The edges of the sheets were fixed with bricks and muds. Afterward the pit was covered with 25 -30 kg of fine sieved fertile soil mixed with carbofuran @1% of total weight of soil and spread uniformly over the surface. Then the water was transferred into the pit up to the height of 25 – 30 cm. Slurry of cow dung (1-2 day old) about 6-7 kg was prepared and poured into the pit. SSP was spread @ 20-25 gm over each tank. Any foreign root and unwanted material over the surface is removed with the help of wire-mesh. Finally the pure fresh Azolla culture is inoculated @ 300-400 gm /m^2 over the surface. To maintain a faster growth, cow slurry (1-2 kg) and SSP of about 10 gm was poured at every 10th day gap. 25 to 30 % of the water was to be replaced with fresh water once in every 15 days to prevent nitrogen build up in the pit. The pit was covered with the long plastic polythene sheet during the night time (plate1) and was removed every morning around 9 am. This method helped a lot for Azolla growth and prevented it from cold stress and dew.

Sample collection and storage

Growth rate of *Azolla pinnata* is very fast and it covered the whole pit within 8- 10 days (Plate 2). The fresh biomass of *Azolla pinnata* is harvested at every 2nd day and washed to remove the extraneous materials from it using wire-mesh and water (Plate 3). The harvested *Azolla pinnata* was dried under shade to remove moisture and then oven dried at 45°C for 48 hrs. The oven-dried sample was ground to 1 mm size and further used for chemical analysis.

Proximate analysis of *Azolla pinnata*

All the feed samples were chemically analysed for the crude protein (CP), Neutral detergent fibre **(**NDF), Acid detergent fibre (ADF), total ash (TA), ether extracts (EE) and moisture according to methods of Association of Official Analytical Chemists [6]. The DM content of Azolla was measured by drying the sun dried sample in a hot air oven at 100°C for a longer period (8-24 hrs.). The total ash content of sample was measured as residue by incineration of samples at 600^{0}C for 3 hours

in muffle furnace.The Crude protein (N X 6.25) of Azolla was analysed using KELPLUS KES 1 2L digestion bench and of Kjel-Tech Nitrogen Distillation Assembly [6]. The ether extract of the sample is estimated by extracting it with petroleum ether using the Soxhlet's apparatus as per procedure of (AOAC 2005). The Nitrogen free extract was calculated using standard conventional Weende's method and the NDF, ADF were determined.

Mineral profiling of *Azolla pinnata* (on percent DMB)

Mineral profile of Azolla was analysed by spectrophotometer and amount was calculated by below given formula.

µg / g = Concentration of mineral in sample solution (mg / L) x Volume made (ml)/ Weight of sample (g)

Results and Discussion

The results of the proximate estimation of sun dried Azolla have been presented in the table 1. The average DM content obtained in the experiment was 9.95 %.The crude protein, ether extract, crude fibre, nitrogen free extract, total ash, acid insoluble ash, NDF, ADF, organic matter and hemicellulose in Azolla (*Azolla pinnata*) were found to be 26.5 %, 3.9 %, 13.45 %, 20.3%, 44.28 %, 39.4 %, 79.7 %, and 13.69 %. The average content of DM recorded was 9.90 ± 0.08%.Comparatively higher DM was reported by (Akhud *et al.,* 2017) who recorded a DM of about 12% in Azolla. The crude protein (CP) content of *Azolla pinnata* ranges from 24.65 to 28.96 with an average value of 26.5±0.08%.Similar or slightly higher value of CP were obtained by various researchers 26.7 %, (Becerra *et al.,* 1995), 28.59 % (Ahirwar and Leela 2012) and 28.24% (Indira *et al.,* 2009). However lower values of CP were reported by other researchers 21.17 % (Sujatha *et al.,* 2013), 21.4% (Alalade and Lyayi 2006), 21.66 %, (Kavya 2014) and 22.5 % (Ashraf and Sharma 2015).

The possible reason for this CP variation may be due to temperature, nutritional content of soil, water quality (pH), pest growth and mineral mixture obtained from external source that may have affected its growth and composition. The crude fibre value obtained in the experiment was 13.45 %. The values obtained were in close agreement with the values obtained by (Balaji *et al.,* 2009, Cherryl 2014). Alalade and lyayi (2006) reported a lower value of 12.7 per cent.

However the higher range of CF values from 15.17 to 19.85 was recorded by (Kavya 2014). This change in the CF values may be due to change in DM content and maturity level of the Azolla that was collected for estimation at different intervals. The ether extract calculated in the present experiment was found to be around 3.9±0.13 %.

The results obtained were similar to the one obtained by (Chatterjee *et al.,* 2013, Alalade and Lyayi 2006, Kumar *et al.,* 2012, Querubin *et al.,* 1986, Ghodake *et al.,* 2012) .However (Buckingham *et al.,* 1978, Arvindraj 2012) recorded higher values of crude fibre i.e. 5.05%, and 4.06% in A. microphylla, respectively. The total ash on DM % basis was recorded to be around 20.3±0.28.The result obtained were similar to the one obtained by (Sharma 2013) on *A.microphylla* recording the value around 20.21 % and (Indira *et al.,* 2009) recorded value around 20.21%.

However the higher value for total ash were obtained by (Querubin *et al.,* 1986) recording 28.70% Slightly lower value for Total ash was obtained by (Alalade and Lyayi 2006).

Table.1 proximate composition of *Azolla pinnata*

Components	*Azolla pinnata*
DM (%)	9.95±.03
OM (% DM)	79.7±0.18
NDF (% DM)	44.28±0.18
ADF (% DM)	39.4±0.06
CP (% DM)	26.5±0.08
EE (% DM)	3.9±0.13
TA (% DM)	20.3±0.28

Table.2 THI during study period (weekly)

Weeks	THI value
0 day	70.10
1 Week	69.20
2 Week	67.30
3 Week	71.30
4 Week	69.40
5 Week	68.10
6 Week	70.10

Table.3 Mineral profiling of *Azolla pinnata* (on percent DMB)

Minerals	Percentage	Ppm
Calcium	1.51	
potassium	2.41	
Manganese		2170
Zinc		230
Copper		4.1
Cobalt		7.10
Iron		1100
Nickel		6.10
Chromium		2.01
Cadmium		0.70

Table.4 *Azolla pinnata* yield during winter season

Parameter	Amount
Area of 1 pit	11*4.5=49.5 ft^2
Area of total 19 pits	49.5*19=940.5 ft^2
Total yield	1809.10kg
***Azolla pinnata* growth period**	90 days
Yield of 1 ft^2 (90days)	1.92 kg
Yield of 1 ft^2 / day	0.021kg

Plate.1

Plate. 2

Plate.3

NDF content (percentage of DM) of *Azolla pinnata* varied narrowly from 44.19 to 45.89 with an average value of 44.28 ± 0.18, which was similar to the values reported by (Chatterjee *et al.,* 2013, Sharma 2013, Kumar *et al.,* 2012) in *A.microphylla* (Indira *et al.,* 2009) recorded significantly greater NDF content of 67.8% for *A.pinnata* (Alalade and Lyayi 2006, Buckingham *et al.,* 1978) recorded lower NDF values (36.88, 40.36 and 39.16 percent).

The ADF content in our present research was 39.4 ± 0.14; comparable to those reported by (Ghodake *et al.,* 2012, Arvindraj 2012) but lower than those reported by (Indira *et al.,* 2009, Alalade and lyayi 2006) in *A.pinnata* and (Querubin *et al.,* 1986) for *A. microphylla*, who reported the value of 66.18%, 47.08% and 44.5% respectively. However, (Chatterjee *et al.,* 2013, Kumar *et al.,* 2012, Sharma 2013) recorded relatively lower ADF values. The level of THI during study period was depicted in Table 2 and and the mean level of Temperature humidity index was below 72 in the range of (68-70).

Mineral profiling of *Azolla pinnata*

Mineral profiling of *Azolla pinnata* (Table 3) depicts it as an alternative feed for livestock as Calcium, phosphorus and Potassium are 1.67%, 0.31% and 2.68% respectively. The mineral profiling of *Azolla pinnata* in our present study was in agreement with the results obtained by (Kavya 2014, Anitha 2016, Anand and Geetha 2007) and the values of mineral profiling by. Yield of *Azolla pinnata* obtained was 1809.10 kg (Table 4) and Yield of 1 ft^2 / day was 0.021 kg. The Yield of *Azolla pinnata* obtained in our experiment was similar to that of (Chatterjee *et al.,* 2013).

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From this study it may be concluded that *Azolla pinnata* is having better mineral profile, sufficient yield per square feet area and thus it can be used as an alternative and sustainable feed for livestock

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